and causes N-type inactivation. We explored the contacts between alpha and beta2 subunits by determining the extent of endogenous disulfide bond formation between cysteines substituted in the extracellular flanks of the two beta2 transmembrane (TM) helices, TM1 and TM2, and in the extracellular flanks of each of the seven alpha TM helices, S0-S6. We found that the extracellular ends of beta2 TM2 and alpha S0 are close and that beta2 TM1 is close to both S1 and S2. At their extracellular ends, TM1 and TM2 are not close to S3, S4, S5 or S6. Beta2 TM1 and TM2 are like pincers on either side of the alpha voltage-sensor domain, S0-S4. In all tested pairs of cysteine-substituted alpha and beta2, we found that disulfide crosslinks favored the closed state, shifting the conductance-voltage curves toward more positive potentials and slowing the kinetics of activation. N-type inactivation, involving three specific beta2 residues in its cytoplasmic, N-terminal segment preceding TM1, was not affected by any of the crosslinking. This is consistent with the above locations of TM1 and TM2 because a minimum of 12 residues, spanning up to 40 Å, allows the three N-terminal inactivating residues to reach the pore (Xia et al., 2003 J. Gen. Physiol. 121:125). The positions of the beta2 TM helices are similar to the locations that we previously reported for beta1 TM1 and TM2 (Liu et al., 2008 PNAS 105:10727). Supported by NIH NS054946.

2449-Pos Board B419

The β2 subunit modulation of BK channels is determined by membrane-spanning and cytoplasmic domains In Slo1

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Ca^{2+} and voltage activated BK channels are composed of pore forming Slo1 subunits. These channels are modulated by various tissue-specific accessory β subunits, which render BK channels the phenotypes necessary for different physiological functions. Here we study Ca^{2+} sensitivity increase in BK channel activation by the β2 subunit, and elucidate the structural domains in Slo1 that determine this modulation. We found that β2ND (β2 with $\underline{NH_2}$-terminus deleted to remove inactivation) (Wallner et al., *PNAS* **96**(7):4137-42, 1999) increased Ca^{2+} sensitivity in mouse Slo1 (mSlo1) but not in drosophila Slo1 (dSlo1). Taking advantage of these differential effects, chimeras of mSlo1 and dSlo1 were studied. When chimeras in the mSlo1 background contained the S0 transmembrane segment and the N-terminal region of RCK1 (regulator of K^+ conductance) termed the AC region (Krishnamoorthy et al., *JGP* **126**(3): 227-41, 2005) from dSlo1, β2ND failed to increase Ca^{2+} sensitivity. When these same regions from mSlo1 were in dSlo1, the channels showed increased Ca^{2+} sensitivity in association with β2ND. Thus, the mouse AC region and S0 segment are necessary and sufficient for the β2 subunit to increase Ca^{2+} sensitivity. Previous studies suggested that each Slo1 subunit contains two different Ca2+ binding sites (Xia et al., *Nature* **418**(6900): 880-4, 2002). To further investigate the β2 subunit modulation, we studied the effect of β2ND with mutations of the binding sites. We found that the effect of β2ND was nearly intact when either site was ablated and was completely destroyed when both sites were mutated. These results suggest that the β2 subunit may affect an allosteric activation pathway that is common to both binding sites, and S0 or the AC region is part of such pathway.

2450-Pos Board B420

The Locations of the Beta4 Transmembrane Helices in the BK Channel

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The large-conductance, Ca^{2+}- and voltage-activated potassium channel (BK) alpha subunit is modulated by one of four types of beta subunits, each imparting unique electrophysiological properties. BK beta4 is expressed in brain. It slows both activation and deactivation, with only small shifts in V_{50}, and confers resistance to block by charybdotoxin and iberiotoxin. In mice, deletion of beta4 causes temporal lobe epilepsy. We explored the contacts between alpha and beta4 subunits by determining the extent of endogenous disulfide bond formation between cysteines substituted in the extracellular flanks of the two beta4 transmembrane (TM) helices, TM1 and TM2, and in the extracellular flanks of each of the seven alphaTM helices, S0-S6. We found that the extracellular ends of beta4 TM2 and alpha S0 are close and that beta4 TM1 is close to both S1 and S2. At their extracellular ends, TM1 and TM2 are not close to S3, S4, S5 or S6. Beta4 TM1 and TM2 are like pincers on either side of the alpha voltage-sensor domain, S0-S4. Crosslinking of beta4 TM2 to S0 further slowed activation and deactivation kinetics, with either no effect on V_{50}, or causing a small hyperpolarizing shift. Thus, crosslinking enhances the predominant effect of beta4 on the transition rates between the activated and deactivated states, with little effect on the free energy differences between these states. Supported by NS054946.

2451-Pos Board B421

Molecular and Functional Expression of the Best2 Ca2 ± activated Cl- Channel in Mouse Submandibular Salivary Gland

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Activation of Cl^- channels in salivary acinar and duct cells is essential for saliva production. Anion efflux through an apical Ca^{2+}-dependent Cl^- channel (CaCC) is the rate limiting step for fluid secretion by acinar cells. The ionic composition of the primary saliva is then modified by salivary ducts. CaCC may support electrolyte reabsorption by duct cells of several types that constitute the duct system. The molecular identity of salivary CaCC is currently under vigorous examination. Here we explored the function of Best2, a member of the Bestrophin family of CaCCs, in the mouse submandibular salivary gland. Heterologous expression of the Best2 transcript in HEK293 cells produced Ca^{2+}-activated Cl^- current with the biophysical and pharmacologic properties that closely resembled the current found in native salivary cells. A recently developed *Best2*$^{-/-}$ mouse where the gene was disrupted by insertion of Lac Z was used to further characterize the role of this channel in the exocrine salivary gland. Even though Best2 expression was abolished, the amplitude and properties of the Ca^{2+}-activated Cl^- current in the acinar cells obtained from Best2-deficient mice were the same as the Ca^{2+}-activated Cl^- current in wildtype cells. Consistent with the observation the fluid secretion rate was not significantly different in *Best2* null mice. *Best2* gene was highly expressed in the duct cells of submandibular glands as revealed by X-gal staining. While, the ionic composition and osmolality of the saliva was not significantly altered in mice lacking Best2, the possibility of the functional compensation has been investigated in duct cells. Granular duct cells failed to present Ca^{2+}-sensitive component of anion conductance. The properties of Cl^- channels in intercalated, striated and excretory duct cells are currently under investigation.

2452-Pos Board B422

Angiotensin Ii Activates Calcium-Dependent Cl- Channels In Human Cardiac Fibroblasts

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This study reports for the first time the presence of chloride channels on the plasma membrane of human cardiac fibroblasts in culture, by means of the iodide efflux and the patch clamp methods. The angiotensin II and the calcium ionophore A23187 activate a chloride conductance that shares pharmacological similarities with calcium-dependent chloride channels already described in other cell types. Using the iodide efflux technique it was shown that Ag II could induce an anionic efflux after binding to AT1 receptors (with an EC50 = 13.8 ± 1.3 nM). Blockade of chloride efflux by calphostin C and KN 62 indicates that this activation is dependent on PKC and/or CaMKII. This calcium-dependent chloride current which is characterized in human cardiac fibroblasts is potentially involved in the secretion by cardiac fibroblasts of growth factors; collagen and pro-inflammatory mediators released in particular pathological conditions.

2453-Pos Board B423

SKA-31, A New Activator of KCa2 And KCa3.1 Potassium Channels, Potentiates the EDHF Response and Lowers Blood Pressure

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Small-conductance (KCa2.1-2.3) and intermediate-conductance (KCa3.1) calcium-activated K^+ channels are critically involved in modulating calcium-signaling cascades and membrane potential in both excitable and non-excitable cells. Activators of these channels constitute useful pharmacological tools as well as potential new drugs for the treatment of ataxia, epilepsy, and hypertension. We here used the neuroprotectant riluzole as a template for the design of KCa2/3 channel activators that are potent enough for *in vivo* studies. Out of a library of 55 benzothiazoles we identified two compounds, SKA-20 (anthra[2,1-*d*]thiazol-2-amine) and SKA-31 (naphtho[1,2-*d*]thiazol-2-amine), which are 10-20 times more potent than riluzole and activated KCa2.1 with EC_{50}s of 430 nM and 2.9 μM, KCa2.2 with EC_{50}s of 1.9 μM, KCa2.3 with EC_{50}s of 1.2 μM and 2.9 μM, and KCa3.1 with EC_{50}s of 115 nM and 260 nM. Likewise, SKA-20 and SKA-31 activated native KCa2.3 and KCa3.1 channels in murine endothelial cells and the more ''drug-like'' SKA-31 (half-life 12 hours) potentiated endothelium-derived hyperpolarizing factor-mediated dilations of carotid arteries from KCa3.1$^{+/+}$ mice but not from KCa3.1$^{-/-}$ mice.

Administration of 10 and 30 mg/kg SKA-31 lowered mean arterial blood pressure by 4 and 6 mmHg in normotensive mice and by 12 mmHg in angiotensin-II-induced hypertension. These effects were absent in KCa3.1-deficient mice. In conclusion, with SKA-31 we have designed a new pharmacological tool to define the functional role of KCa2/3 channel activation *in vivo*. The blood pressure lowering effect of SKA-31 suggests KCa3.1 channel activation as a new therapeutic principle for the treatment of hypertension.

2454-Pos Board B424

Molecular Action Of CFTR Potentiators On The Kca3.1 Channel

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Airway epithelial cells are the site of Cl- secretion through the cystic fibrosis transmembrane regulator (CFTR). Cystic fibrosis (CF) is a fatal genetic disease caused by mutations in CFTR. The most frequent mutation consists of a deletion of the phenylalanine at position 508 (ΔF508-CFTR) that impairs protein maturation and alters channel gating. In the last years, several small molecules were identified by high throughput screening that could restore ΔF508-CFTR function. Compounds addressing ΔF508-CFTR gating defects are referred to as potentiators and have been documented to increase the activity of ΔF508-CFTR to a level similar to wild-type CFTR. The basolateral K+ channel KCa3.1 has been documented to play a prominent role in establishing a suitable driving force for CFTR-mediated Cl- secretion in airway epithelial cells. Thus, in a global approach of transepithelial transport, the research for physiologically relevant ΔF508-CFTR potentiators should also consider their effects on the KCa3.1 channel. A characterization of the effect of different ΔF508-CFTR potentiators on the KCa3.1 channel was undertaken using inside-out patch clamp measurements on cDNA injected *xenopus* oocytes and on transformed HEK-293 cells that express the KCa3.1 channel. In this work we present preliminary results on the effects of different ΔF508-CFTR potentiators on KCa3.1. Our inside-out patch-clamp measurements show that VRT-532 has a state independent inhibitory effect on KCa3.1, but very little action on the V282G mutant of KCa3.1, which is constitutively active. In contrast, CBIQ succeded to activate KCa3.1, through a mechanism likely to involve an action on the channel gate. These effects were observed at concentrations known to activate ΔF508-CFTR. Supported by CCFF.

2455-Pos Board B425

Mechanism of Benzofuroindole-induced Potentiation of BK_{Ca} channel

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In our previous studies, we reported that the activity of the large-conductance calcium-activated potassium channels (BK_{Ca} channel) could be strongly potentiated by certain derivatives of benzofuroindole scaffold (Gormemis *et al.*, 2005; Ha *et al.*, 2006). Here, we characterized the mechanism of action of these compounds. Benzofuroindoles potentiated the channel by shifting its conductance-voltage relations toward the more negative direction without affecting its voltage sensitivity. This drug was proven to act on the alpha-subunit of the channel (Slo1) from the extracellular side. The dose-response curve of the drug could be well fitted with the Hill coefficient close to 1. While the apparent affinity of the drugs was not affect by tetraethyl ammonium, a channel-blocking quaternary ammonium, the co-treatment of charybdotoxin significantly decreased the potency of the compounds, suggesting the potential competition between the drug and the peptide blocker. Guided by these results, we performed the mutagenesis studies on the outer vestibule of the BK_{Ca} channel in order to localize the drug binding site. Among one deletion and 19 alanine substitutions, four mutant channels showed significantly smaller shifts in their conductance-voltage curves by the drug treatment compared to the wild-type. Since these mutations were clustered at the 'turret' region of the channel, benzofuroindole derivatives may stabilize the open conformation of BK_{Ca} channel by binding to this area.

2456-Pos Board B426

Energetic Performance is Improved by Specific Activation of K+ Fluxes through KCa Channels in Heart Mitochondria

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K^+ movement across mitochondrial membranes is involved in volume regulation and may play a role in cardioprotection. The Ca^{2+}-dependent K^+ (KCa) channel has been proposed as a contributor to mitochondrial K^+ uniport activity, but its functional role is not well understood. To investigate the impact of KCa channels on mitochondrial energetics, we measured K^+ fluxes in parallel with $\Delta\Psi_m$ and light scattering in isolated mitochondria from guinea pig hearts. We first analyzed the role of different anions on K^+ fluxes. Mitochondria loaded with the K^+-sensitive fluorescent probe PBFI were incubated with 5mM glutamate-Na^+/malate-Na^+ in isotonic sucrose medium and subjected to pulses containing different concentrations of KCl, KAc or KH_2PO_4. K^+ fluxes saturated at $\approx$10mM regardless of the anion, attaining maximal rates (nmol K^+/min/mg) of 172 ± 17 (KCl), 84 ± 2.4 (KAc), and 74 ± 3.8 (KH_2PO_4), with similar $K_{0.5}$ in all three cases. We then analyzed the effect of NS11021, a novel activator of KCa channels, on the maximal K^+ uptake rate. In the presence of KH_2PO_4 or KAc, 20-50nM of NS11021 increased mitochondrial volume and K^+ flux by ~2.5-fold whereas KCl increased K^+ uptake by 30% with little change in volume. $\Delta\Psi_m$ was minimally affected in this concentration range. The NS11021 effect was blocked by 200nM charybdotoxin, a KCa channel blocker. At 50nM NS11021, the respiratory control ratio of the mitochondria increased 2.5-fold in the presence of KH_2PO_4, but not KCl, indicating that a regulatory volume increase is required to improve oxidative phosphorylation. At higher concentrations of the compound ($\geq 1\mu M$) substantial effects on $\Delta\Psi_m$ and state 4 respiration were observed, which were not inhibited by Chtx. The findings indicate that activation of K^+ fluxes through KCa channels, coupled with swelling without loss of $\Delta\Psi_m$, improves mitochondrial energetic performance.

Cyclic Nucelotide-gated Channels

2457-Pos Board B427

Structural and Energetic Analysis of the Cyclic nucleotide binding domain from the MlotiK1 Potassium Channel

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MlotiK1 is a cyclic nucleotide-dependent ion channel which contains an intracellular C-terminal cyclic nucleotide binding domain (CNB domain). We have used X-ray crystallography to determine several different structures of the MlotiK1 CNB domain structures in the bound and unbound state. In combination, the five MlotiK1 CNB domain structures provide a unique opportunity for analyzing, within a single protein, the structural differences between the *apo* and bound states and the structural variability within each state. With this analysis as a guide, we have probed the nucleotide selectivity and importance of specific residue side chains in ligand binding and channel activation. These data help to identify ligand-protein interactions that are important for ligand-dependence in this channel and more globally in the class of nucleotide-dependent proteins.

2458-Pos Board B428

Enhancement of Voltage Sensitivity of a cGMP-gated Channel

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Activity of cyclic nucleotide-gated (CNG) cation channels underlies signal transduction in vertebrate visual receptors. These channels must be primarily activated by the binding of cGMP so that the activity of these highly specialized receptor channels be controlled by ligands in a finely graded manner required for transducing sensory stimuli of varying intensity. Significant voltage sensitivity of the channels would generate voltage-driven positive feedback and thus reduce the signal-transduction sensitivity. Indeed, the CNGA1 channel is only modestly voltage sensitive in low cGMP concentrations, and the voltage sensitivity vanishes with increasing cGMP concentration. We have found that loosening the attachment of the selectivity filter to the surrounding ''pore shell'' dramatically increases the channel's voltage sensitivity, which is independent of the positively charged residues in S4. Thus, proper attachment of the selectivity filter is essential to avoid significant, adverse voltage sensitivity in these channels.

2459-Pos Board B429

Lidocaine Inhibition of HCN1 Channels is Fast, Voltage-dependent and Reversible

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Hyperpolarization-activated Cyclic Nucleotide-modulated (HCN) channels underlie the funny current or the hyperpolarization-activated current (If or Ih), which is important in regulating excitability in the neurons of the central nervous system and in the conduction tissue of the heart. There are four mammalian isoforms of HCN channels (HCN1-4), each exhibiting different kinetics, voltage dependence, and amounts of inward, time-dependent current (If). Lidocaine, a local anesthetic and antiarrhythmic drug, has been shown to inhibit HCN-mediated currents in the rabbit sinoatrial (SA) node, which expresses various HCN isoforms. Previously, we showed that lidocaine, at concentrations ranging from 20 to 200 μM, inhibits mouse HCN1 channels, but the rate and extent of both